UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

NOV 0 2 2004

MEMORANDUM

SUBJECT: Science Review of product performance studies for registration of B2E-04, EPA Registration No. 75318-G, containing 4.25 % (S)-Methoprene (CAS# 65733-16-6) as active ingredient.
DP Barcode: Not found. Decision No. Not found.

FROM: Clara Fuentes, Ph.D., Biologist
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511C)

THROUGH Angela Gonzales, Biologist
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511C)

TO: Mari Duggard, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511C)

ACTION REQUESTED

B2E Biotech LLC. submitted 2 product performance studies designed to evaluate efficacy of B2E-04, containing 4.25 % (S)-Methoprene, against mosquitoes in support of product registration.

RECOMMENDATIONS AND CONCLUSIONS

1. Data derived from the submitted studies do not support lasting efficacy of the product for 30 days as stated on the product label.

2. Must resolve discrepancy between data reported on page 6 and Table 1 of MRID 461150-06 for average percent IEs of larvae introduced 28 and 35 days post treatment. The text on page 6 reports average IE 40 and 60 percent of larvae introduced 28 and 35 days post-treatment, respectively. This information appears inverted in Table 1, where average IE for larvae introduced 28 and 35 days post harvest is 60 and 40 percent, respectively.

STUDY SUMMARY

MRID 461150-05 **Unacceptable.**

This study summarizes product performance data from MRID 461150-06, discussed below, and from study number 071902AP, which is not available to the reviewer. Study # 071902AP compares efficacy of the product with that of Altosid Pellets (4.25% S-methoprene) against natural populations of *Culex tinnaefasciatus* in outdoors microcosms. Both products were applied at equal rate of 10 lbs/A (193 g a.i/A). For larvae introduced on treatment day and 4 days later, B2E-04 outperformed Altosid pellets, inhibiting emergence by 84 and 80 percent compared to 43 and 63 percent for Altosid, respectively. No specific average percent of IE by either product was provided for other treatments.

MRID 461150-06 **Acceptable for control of flood water mosquitoes up to 7 days.**

The study evaluated efficacy of B2E-04 (4.25% S- methoprene) against *Psorophora columbiae* applied at a rate of 2.5 lbs of product/A to six 625 sq. ft. experimental flooded rice plots. The product completely inhibited emergence (IE) of *P. columbiae* larvae up to 7 days post-treatment. Efficacy declined after that: IE of larvae introduced 14 and 21 days after treatment averaged 73 and 83 percent, respectively. The text reports averages of 40 and 60 percent for IE of larvae introduced 28 and 35 days post-treatment, respectively. This information appears inverted in Table 1, where average IE for larvae introduced 28 and 35 days post treatment is 60 and 40 percent, respectively.

BACKGROUND AND REVIEWER COMMENTS

Lasting efficacy of the product is variable with rates and mosquito's species. The data derived from study MRIDs 461150-06 does not support the range of application rates recommended on the label for the control of flood water mosquitoes up to 30 days. Based on the 95% minimum population reduction recommended by OPPTS guideline 810.3400, the product, B2E-04, when applied at a rate of 2.5 lbs/A (the lowest rate recommended on the label) under the experimental conditions described in the study, was effective against *P. columbiae* larvae up to 7 days post-treatment. Study # 071902AP (unavailable to the reviewer) fails to provide numeric results comparing the lasting performance of B2E-04 with that of Altosid for tests beyond 4 days post-treatment.

DATA EVALUATION RECORD

ISOPROPYL (2E,4E,7S)-11-METHOXY-3,7,11-TRIMETHYL-2,4-DODECADIENOATE (B2E-04)

STUDY TYPE: Product Performance, OPPTS 810.3400

MRID 46115005

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Work Assignment 04-69

Primary Reviewer:
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Signature: Eric B. Lewis
Date: SEP 29 2004

Secondary Reviewers:
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Date: SEP 29 2004

Robert H. Ross, M.S., Group Leader

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Date: SEP 29 2004

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: SEP 29 2004

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Product Performance, OPPTS 810.3400
MRID NO:	46115005
DP BARCODE:	75318-G
TEST MATERIAL:	B2E-04
STUDY NO:	B09/03/0043A
SPONSOR:	B2E Biotech LLC, 500 Mamaroneck Ave., Harrison, NY 10528
TESTING FACILITY:	B2E Biotech LLC, 500 Mamaroneck Ave., Harrison, NY 10528
TITLE OF REPORT:	B2E-04 Product Performance Summary and Bridging Data
AUTHOR:	Sjogren, R.D.
STUDY COMPLETED:	September 29, 2003
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE	A signed GLP statement was included. The study was not subject to GLP standards.
STUDY SUMMARY:	MRID 46115005 summarizes product performance data from MRID 46115006 and Study Number 071902AP, which were submitted to support registration of B2E-04 for control of mosquitoes. In MRID 46115006, B2E-04 (a.i.. 4.25% S-methoprene) was applied at a rate equivalent to 2.5 lbs of product/A to experimental flooded rice plots. Bioassay containers of late third/early fourth stage *Psorophora columbiae* mosquito larvae were installed shortly after treatment, and weekly thereafter for the next five weeks, and monitored for mortality and adult emergence. B2E-04 inhibited adult emergence by 100% up to 7 days post-treatment, compared to 1.6% in untreated controls. Inhibition of emergence for larvae introduced 14 and 21 days after treatment averaged 73% and 83%, respectively, compared to 7% and 0%, respectively, for controls. Contradictory information was presented for inhibition rates on days 28 and 35 post-treatment. In Study Number 071902AP, efficacy of B2E-04 was compared to that of Altosid Pellets (4.25% S-methoprene) against natural populations of *Culex quinquefasciatus* in outdoor microcosms. Both products were applied at a rate of 10 lbs/A (193 g a.i./A). For larvae introduced on the treatment day or day 4 post-

treatment, B2E-04 significantly outperformed Altosid Pellets, inhibiting adult emergence by 84% and 80%, respectively, compared to 43% and 63% for Altosid. For larvae introduced on post-treatment days 7, 11, and 14, inhibition was essentially the same by both products. For larvae introduced on day 18, B2E-04 provided significantly greater inhibition than Altosid (numbers not provided). For larvae introduced on days 21 and 25, there was no difference in the two products (numbers not provided). Inhibition of emergence (IE) has become the standard for evaluating insect growth regulator larvacides. After several tests and replications, dose-response lines can be established by plotting %IE against concentration, and LC_{50} and LC_{90} values can then be obtained from the dose-response lines or from computer regression analysis. While IE has become the standard method to evaluate methoprene efficacy, it only partially measures the impact of methoprene treatments. Sublethal methoprene exposure can produce numerous physiological imbalances that, while not measured by IE, negatively impact the ability of mosquito populations to survive.

CLASSIFICATION: Supplemental

Test Material

The test material was B2E-04 (a.i., 4.25% S-methoprene), a pellet formulation.

Introduction

MRID 46115005 summarizes product performance data from two studies (MRID 46115006 and study number 071902AP, which was not available to the reviewer) to support registration of B2E-04 for control of mosquitoes. MRID 46115005 also includes a chart of relative mosquito species susceptibility to methoprene and an appendix of published literature to support the methods used in the two studies.

Results Summary

MRID 46115006 evaluated a single hand-applied B2E-04 treatment of 16.28 g of product to 625 ft^2 flooded rice plots (2.5 lbs product/A). The test consisted of three replicates over time, and three untreated plots served as controls. B2E-04 achieved complete inhibition of emergence (IE) of *Psorophora columbiae* larvae introduced at the time of treatment or at seven days post-treatment. The IE of larvae introduced 14 or 21 days post-treatment was 73% and 83%, respectively, compared to 7% and 0% for controls. The IE decreased to 40% for larvae introduced 28 days post-treatment, then increased to 60% for larvae introduced 35 days post-treatment.

According to the study author, Study Number 071902AP compared the efficacy of B2E-04 with that of Altosid Pellets (4.25% S-methoprene) against natural populations of *Culex quinquefasciatus* in outdoor microcosms. Both products were applied at a rate of 10 lbs/A (193 g a.i./A). For larvae introduced on treatment day or day 4 post-treatment, B2E-04 significantly ($p \leq 0.05$) out-performed Altosid Pellets, with an IE of 84% and 80%, respectively, compared to 43% and 63% for the Altosid. For larvae introduced on post-treatment days 7, 11, and 14, the IE was essentially the same for both products. For larvae introduced on day 18, B2E-04 provided significantly greater IE than Altosid (numbers not provided). For larvae introduced on days 21 and 25, there was no difference in the two products (numbers not provided).

Methodology

The study author states that inhibition of emergence (IE) has become the standard for evaluating insect growth regulator larvacides and attached to MRID 46115005 published literature that describes the procedures used in the submitted study. Replicated treatments are used to determine the relative biological activity of insect growth regulators (IGRs), and their overall effectiveness is expressed in terms of inhibition of emergence (IE) over a range of concentrations. After several tests and replications, dose-response lines can be established by plotting %IE against concentration, and LC_{50} and LC_{90} values can then be obtained from the dose-response lines or from computer regression analysis. These values provide a relative activity level that can be compared to that of other compounds.

The study author states that while this method has become the standard method to evaluate methoprene efficacy, it only partially measures the impact of methoprene treatments. In some cases, sublethal effects may be as important as the acute toxic effect in reducing pest numbers. Sublethal methoprene exposure can produce numerous physiological imbalances that, while not measured by IE, negatively impact the ability of mosquito populations to survive. These may include reduction in the number of eggs produced; reduced egg viability; aberrant egg development; control of some species in the larval, as well as the pupal stage; longer larval development times; diminished adult blood-feeding success; and shorter life spans.

Microcosms are used rather than field tests because they overcome the problems associated with field site variability, are highly reproducible, and allow dependable statistical analysis of the test data. Microcosms also allow similar test conditions (i.e., identical nutrient levels, water quality, depth, temperature); precise administration of treatment doses; and comparison of treatments with untreated controls.

Study Author's Conclusions

The study author concluded that, based on these results and a chart of mosquito species susceptibility relative to that of *C. quinquefasciatus* (Figure 1 of MRID 46115005, compiled from published data), B2E-04 label dosage rates are 2.5 to 10 lbs/A (48.24 to 113.5 g a.i./A). The low rate may be used for floodwater mosquitoes (*Aedes, Ochlerotatus, and Psorophora*) and shallow water (<1 foot deep). Higher application rates should be used for semi-permanent water mosquito habitats (*Anopheles, Coqullettidia, Culex, Culiseta, and Mansonia*) and deeper water (>1 foot deep) or to make pre-hatch applications to known mosquito breeding sites pre-flood. The study author also concluded that based on the published literature attached to the study, IE assesses only part of the impact S- methoprene treatment has on immature mosquito populations.

Reviewer's Conclusions

The reviewer does not agree with the study author's conclusion, since it is based in part on the conclusion in MRID 46115006 that B2E-04 was efficacious for 21 days. Based on the 95% minimum population reduction recommended by OPPTS 810.3400, B2E-04 applied at a rate of 2.5 lbs/A (the minimum label rate) was efficacious against larval emergence of *Ps. columbiae* for only up to 7 days post-treatment. However, the product label states that under typical environmental conditions, one application will provide control for 30 days. Study Number 071902AP, which compared the efficacy of B2E-04 with that of Altosid Pellets, was not available to the reviewer.

DATA EVALUATION RECORD

ISOPROPYL (2E,4E,7S)-11-METHOXY-3,7,11-TRIMETHYL-2,4-DODECADIENOATE (B2E-04)

STUDY TYPE: Product Performance, OPPTS 810.3400

MRID 46115006

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
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Work Assignment 04-69

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Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Product Performance, OPPTS 810.3400
MRID NO:	46115006
DP BARCODE:	75318-G
TEST MATERIAL:	B2E-04
STUDY NO:	S09-03-0043B
SPONSOR:	B2E Biotech LLC, 500 Mamaroneck Ave., Harrison, NY 10528
TESTING FACILITY:	Rice Research and Extension Center, University of Arkansas, Stuttgart, AR
TITLE OF REPORT:	Assessment of B2E-04 with *Psorophora columbiae* Larvae
AUTHOR:	Meisch, M.V., and D.A. Dame
STUDY COMPLETED:	August 27, 2002
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE	A signed GLP statement was included. The study met GLP requirements with the following exceptions: there was no QA audit; the stability, characterization, identity, and verification of the test substance concentration as received and tested were the responsibility of the study sponsor; signatures of individual research assistants were not obtained; and B2E Biotech will archive all signed reports and protocols.
STUDY SUMMARY:	B2E-04 (a.i., 4.25% S-methoprene) was applied at a rate equivalent to 2.5 lbs of product/A to experimental flooded rice plots. Bioassay containers of late third/early fourth stage *Psorophora columbiae* mosquito larvae were installed shortly after treatment, and weekly thereafter for the next five weeks, and monitored for mortality and adult emergence. B2E-04 inhibited emergence by 100% up to 7 days post-treatment, compared to 1.6% in controls. Inhibition of emergence for larvae introduced 14 and 21 days after treatment averaged 73% and 83%, respectively, compared to 7% and 0%, respectively, in controls.

Contradictory information was presented for inhibition rates on days 28 and 35 post-treatment.

CLASSIFICATION: Unacceptable

Test Material

The test material was B2E-04 (a.i., 4.25% S-methoprene), a pellet formulation.

Test Methods

Six 625 ft^2 experimental plots were established in Stuttgart, AR and planted in rice. Each plot consisted of the rice pan surrounded by an open perimeter ditch (about 2 ft wide) and bordered by a levee also planted in rice. During an approximate three-month period, three plots received B2E-04 treatment, and three served as untreated controls. The plots were initially flooded so the pan was covered with 2 to 4 inches of water, and the ditches contained 8 to 10 inches of water. At study start (June, 2003) the rice was 10 to 14 inches high and uniformly dense in the pan. At the time of the last replicates (mid to late July), the rice was 16 to 20 inches high. During the test, the plots were flooded to the original water levels with untreated water weekly, as needed.

B2E-04 pellets (16.28 g/plot) were uniformly applied by hand at an application rate equivalent to 2.5 lbs of product/A (80 ppb). Two screened floating bioassay containers, each containing 10 locally-collected late third/early fourth stage *Psorophora columbiae* larvae, were placed in the ditch of each plot shortly after treatment, and weekly thereafter for the next five weeks. Each container was monitored daily for mortality and adult emergence, at which time adults and pupal exuviae were removed. Monitoring continued until all individuals in the cages had emerged or died.

Results Summary

Results are summarized in Table 1. B2E-04 inhibited emergence by 100% up to 7 days post-treatment, compared to 1.6% in controls. Inhibition of emergence of larvae introduced 14 and 21 days after treatment averaged 73% and 83%, respectively, compared to 7% and 0%, respectively in controls. Page 6 and Table 1 of MRID 46115006 are contradictory for inhibition rates on days 28 and 35 post-treatment; the text gives 40% and 60%, respectively, and the table gives 60% and 40%, respectively.

TABLE 1. Percent inhibition of larval emergence of *Ps. columbiae* by B2E-04 in flooded rice plots

Treatment	Replicate	Percent emergence inhibition of larvae installed on Day					
		0	7	14	21	28	35
B2E-04	1	100	100	85	90	50[b]	40
	2	100	100	75	100[a]	40[b]	40
	3	100	100	60	60[b]	90[c]	40
	Mean	100	100	73	83	60	40
Control	1	0	5	5	0	0	0
	2	0	0	10	0	0	0
	3	5	0	5	0	0[c]	0
	Mean	1.6	1.6	6.6	0	0	0

[a]Based on one container; the other one disappeared
[b]Based on one container due to insufficient numbers of appropriately aged larvae
[c]Based on one container of 5 larvae due to insufficient numbers of appropriately aged larvae
Data from p. 7, MRID 46115006

Study Authors' Conclusions

The study authors concluded that by day 28 post-treatment B2E-04 had become ineffective at inhibiting emergence of *Ps. columbiae*.

Reviewer's Conclusions

Based on the 95% minimum population reduction recommended by OPPTS 810.3400, B2E-04 applied at a rate of 2.5 lbs/A (the minimum label rate) was efficacious against larval emergence of *Ps. columbiae* up to 7 days post-treatment. However, the product label states that under typical environmental conditions, one application will provide control for 30 days.

13544

R143942

Chemical:	S-Methoprene
	PC Code: 105402
HED File Code:	41600 BPPD Other
Memo Date:	*11/2/2004*
File ID:	00000000
Accession #:	000-00-9002

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5/2/2007